IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.:		09/976,961	Confirmation No.: 3007
Applicant	:	Keith L. Black et al.	
Filed	:	October 12, 2001	
Title		Method for Inducing Selective Cell Death of Malignant Cells by Activation of Calcium-Activated Potassium Channels	
Examiner	:	Brandon J. Fetterolf	
Art Unit	:	1642	
Docket No.	:	11298.105011 (IMN 300US)	
Customer No.	:	20786	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Keith L. Black, M.D.

1. My name is Keith L. Black.

2. I received my Bachelor of Science (B.S.) in Biomedical Science with distinction from the University of Michigan at Ann Arbor in 1978.

3. I received my Medical Degree (M.D.) with distinction from the University of Michigan at Ann Arbor in 1981.

4. From 1981-1987, I completed my internship in General Surgery and then a residency in Neurological Surgery at the University of Michigan Medical Center.

5. From 1987-1991, I was an Assistant Professor in the Division of Neurosurgery at UCLA Medical Center in Los Angeles, California. I was promoted to Associate Professor in 1991, and full Professor in 1994.

6. In 1988, I became the Head of Neuro-Oncology at the UCLA Medical Center.

7. In 1992, I became the Ruth and Raymond Stotter Chair of the Department of Surgery at the UCLA Medical Center.

8. In 1995, I became a Professor of Neurology at the UCLA Medical Center.

9. In 1996, I became the Head of the UCLA Comprehensive Brain Tumor Program.

10. From 1997 until present, I have been affiliated with the Cedars-Sinai Medical Center in Los Angeles, California. At Cedars-Sinai, I serve as the Ruth and Lawrence Harvey Chair in Neuroscience, the Director of the Comprehensive Brain Tumor Program, the

Director of the Division of Neurosurgery and the Director of the Cedars-Sinai Neurological Institute.

11. I am presently a Director of, and a scientific and medical advisor to Imagine Pharmaceuticals, Inc., a company that develops agents that open and close the blood-brain barrier.

12. I have been the recipient of numerous grants, including 8 NIH grants and a Robert Wood Johnson Foundation Faculty Award Grant.

13. I have received numerous awards and recognitions for my scientific and surgical expertise.

14. I have been an author on more than 250 scientific and medical publications.

15. I have been on the editorial board of the following scientific and medical journals: UCLA Cancer Trials; Critical Reviews in Neurosurgery; Perspectives in Neurological Surgery; the Journal of Neuro-Oncology; Neurological Research; the Journal of Radiosurgery; Gene Therapy and Molecular Biology; Neurosurgery Quarterly. I am also the editor of the internet publication Neuroscience Medicine and Technology (Net).

16. I am a named inventor on U.S. Patent Application No. 09/976,961 (the '961 application). I have read and understood this application.

17. I understand that the Examiner has rejected the pending claims in the '961 application on the grounds that they are not enabled by the specification. The '961 application teaches that activators of calcium-activated potassium channels (K_{CA} channels) can selectively induce apoptosis in malignant cells, such as glioma cells, whether in vitro or in vivo.

18. Claim 1 of the '961 application is directed to a method of selectively inducing apoptosis of a malignant cell comprising administering to a malignant cell a calcium-activated potassium channel activator in an amount sufficient to induce apoptosis of the cell.

The Method is Enabled for Inducing Apoptosis or Inhibiting the Proliferation of Malignant Cells or Tumors Generally

19. I understand that the Examiner has acknowledged that the pending claims are enabled for a method for inducing apoptosis or inhibiting the proliferation of a glioma cell or tumor. However, the Examiner suggests that it would be unpredictable whether the methods of the present invention could be used to induce apoptosis or inhibit proliferation or growth of *any and all* malignant cells or tumors. Based on my years of research, I disagree for the following reasons.

20. The invention is founded on our discovery that malignant cells and tumors over-express K_{CA} channels. The fact that malignant cells have high levels of expression of these K_{CA} channels relative to normal cells is important to the selective induction of apoptosis.

21. Our results further confirm that when an activator of K_{CA} is administered to malignant cells, the over-expressed K_{CA} channels allow selective induction of apoptosis.

22. Prior to our discovery, mechanisms of induction of apoptosis had been studied in several different cell types and under various physiological conditions. It had been speculated that some (hypothetical) apoptotic mechanisms may be mediated by the activity of certain types of potassium channels (e.g., Kv1.3 voltage gated potassium channels). However, the contrary and varied effects observed in studies indicated that different potassium channels might play different and specific mechanistic roles in apoptosis, if they played any role at all. No role for K_{CA} channels had been suggested.

23. It was not until we performed our experiments that it was discovered that K_{CA} expression is more abundant in neovasculature and malignant cells compared to normal tissue. The immunohistochemical results from glioma-bearing rat brain sections were described in the '961 application and shown to be consistent with results showing that activation of K_{CA} channels by a K_{CA} activator (NS-1619) selectively induced apoptosis in malignant cells compared to normal cells.

24. We have performed experiments in our laboratory to establish that K_{CA} channels are also over-expressed on metastatic brain tumors of diverse origin. The specific methodology employed in these experiments follows.

25. Formalin-fixed and paraffin-embedded tissue sections from six human subjects with lung or breast cancer metastases to the brain (3 samples of each) were obtained from the department of pathology at Cedars-Sinai Medical Center. The tissue sections were previously viewed and diagnosed by a pathologist (Dr. William Yong). For optimal detection of K_{CA} channel expression in metastatic brain tumor tissue which had been subjected to formalin and paraffin treatment, the tissue sections were deparaffinized, rehydrated, and subjected to an antigen retrieval process by steaming the glass slides in 0.01 M sodium citrate buffer for 10 min. The slides were then rinsed in PBS, incubated with 0.3% hydrogen peroxide for 10 minutes, then blocked with 1% calf serum for 1 hour. Primary antibody, consisting of a goat anti-MaxiKα (1:200, Santa Cruz), was applied to the slides followed by biotinylated anti-goat IgG (1:1000; Jackson). Slides were washed and incubated with avidin-peroxidase complex (Vector Laboratories) for 1 hour and developed with 3,3'-diaminobenzidine (DAB) solution. The stained sections were counterstained with hematoxylin solution followed by dehydration and glass covers applied. Slides were viewed and photographed under bright field conditions using a Zeiss microscope.

26. Exhibit A (unpublished data) shows that metastasized brain cancer cells originating from breast and lung cancers have high levels of K_{CA} channels. The immuno-staining results show that K_{CA} channels are highly expressed on tumor cells and in a few micro-vessels within the tumor mass in all six metastatic brain tumor specimens. Approximately 60-70% of the cells in the metastatic breast cancer specimens were positive, while approximately 50% of the cells in the lung cancer specimens were positive. No detectable staining was observed in the brain tissue peripheral to the tumor periphery. While present

in all of the tumor tissue analyzed, the level of K_{CA} channel expression is variable among the different patient samples. These differences are common and relate to density of expression of the antigen as well as differences in tissue biopsy handling.

27. Based on the data described above, and our extensive experience in this field, I conclude that we have discovered a method to induce apoptosis or inhibit proliferation or growth of a wide range of malignant cells and tumors. This method gives hope to many patients suffering from presently incurable tumors and disorders.

28. I declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing thereon.

Date: 5-12-2005

Keith L. Black, M.D.

Index of Exhibits

Exhibit A	KCA channel expression on metastatic brain and lung cancer cells